PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 3 :
G01N 33/54

A1

(11) International Publication Number: WO 83/ 04313

(43) International Publication Date: 8 December 1983 (08.12.83)

(21) International Application Number: PCT/US83/00781

(22) International Filing Date: 20 May 1983 (20.05.83)

(31) Priority Application Number: 84843/82

(32) Priority Date: 21 May 1982 (21.05.82)

(33) Priority Country: JP

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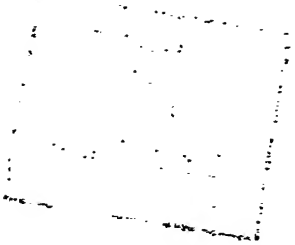
(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, JP, LU (European patent), NL (European patent), NO, SE (European patent), US.

Published
With international search report.

(54) Title: HUMAN-HUMAN HYBRIDOMAS FOR NEOPLASMS

(57) Abstract

Novel hybridomas, human monoclonal antibodies, and their uses. Specifically, CLNH5 is a human-human hybridoma which secretes IgM monoclonal antibodies specific for cervical cells of carcinomas. The monoclonal antibodies can find use in therapy and diagnosis, both in vitro and in vivo.



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HUMAN-HUMAN HYBRIDOMAS FOR NEOPLASMS

BACKGROUND OF THE INVENTION

Field of the Invention

The mammalian immune system has a matchless ability to produce molecules with specificity and avidity for a particular spatial and polar structure, as may be found with sequences of amino acids and sugars. For a long period of time, one was dependent upon producing antibodies employing the immune system in vivo. The resulting polyclonal antibodies demonstrated high specificity for a specific antigen, but could not discriminate between various sites on the antigen and, furthermore, were a mixture of antibodies of varying specificity and avidity. thus, one observed the averaging over the entire composition and not the properties of a specific antibody.

With the seminal discovery by Milstein and Kohler, one can now produce homogeneous compositions of antibodies by fusing a B-lymphocyte with a myeloma cell to produce a cell referred to as a hybridoma. For the most part, the use of this technology has been limited to mouse cells, where stable myeloma lines have served as fusion partners to provide stable hybridomas which can be produced with high efficiency and are capable of being maintained as productive entities over long periods of time. Higher organisms, particularly humans, have proven to be much more intractable in developing fusion partners and hybridomas. However, in 1980, the first human fusion partner was reported by Drs. Olsson and Kaplan and since that time, an additional few human fusion partners have been reported. Nevertheless, the preparation of hybridomas by human-human crosses has remained difficult due to problems of efficiency in fusion, culturing the cells, and maintaining their productive capabilities. However, because of the many advantages of having human hybridomas which produce antibodies allogenic to a

human host, particularly for in vivo applications, human hybridomas remain of great interest. In other instances, even with the difficulties encountered with human-human crosses, the human hybridoma may be preferable to a heterogeneic cross, where the resulting hubridoma may lose the genetic information for the monoclonal antibodies after a number of passages.

One of the areas of interest for the use of monoclonal antibodies is in diagnosing and treating cancer. Monoclonal antibodies for these purposes desirably are specific for a particular type of cancer or subset of cancers, rather than being specific for a particular host cancer cell. it is therefore desirable to develop monoclonal antibodies which can be used in the diagnosis and treatment of human cancers.

Description of the Prior Art

Nowinski et al., Science (1980) 210:537-539 describe human monoclonal antibodies against Forssman antigen. Corce et al., Nature (1980) 288:488-489 describe human hybridomas secreting antibodies to measles virus. Olsson and Kaplan, PNAS USA (1980) 77:5429-5431 describe human-human hybridomas producing monoclonal antibodies of predefined antigenic specificity as well as the fusion partner employed for production of the antibodies. See also copending application Serial No. 247,652, filed March 26, 1981.

Schlom, PNAS USA (1980) 77:6841-6845 describes monoclonal antibodies for breast cancer and Sikora, Brit. J. of Cancer (1981) 43:696 describes separating in situ lymphocytes from a cancer providing antibodies specific for the cancer. In the Proceedings of the 15th Leukocyte Culture Conference, Parker and O'Brien, eds., Wiley Interscience, N.Y., Dec. 5-10, 1982, the subject hybridoma is described. This abstract is incorporated herein by reference.

SUMMARY OF THE INVENTION

Lymphocytes derived from a neoplastic human host are immortalized by fusion with human fusion partners to provide human x human hybridomas secreting monoclonal antibodies specific for a neoplastic cell. Particularly, monoclonal antibodies specific for solid tumor cells such as cervical cancer cells are provided for use in diagnostics and therapy.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Human monoclonal antibodies specific for neoplastic cells from solid tumors are obtained from human x human fusions employing B lymphocytes, e.g., from lymph nodes draining a solid tumor. Particularly, lymph nodes are selected which appear to be active based on necrosis of tumor cells in the vicinity of the lymph node in an immunocompetent host.

The draining lymph node(s) may be isolated in conjunction with a variety of human tissue, e.g., cervix, mammary, colon, lungs, prostate, skin, etc. Of particular interest are lymph nodes from the spinal area.

The fusion partner may be any convenient immortalized B-cell, which does not secrete immunoglobulins, individual chains or fragments thereof, can be selected against, as with HAT medium, and desirably has a high fusion efficiency. Illustrative fusion partners are UC729-6, J-4 (SKO-007), and GM1500 6TG-A12.

The fusions may be performed as described in the literature employing PEG1500 as fusogen, plating the cells in HAT medium in a plurality of wells and then screening supernatants in the viable cell wells for antibodies of interest. Wells positive for reactivity are then cloned by limiting dilution and expanded.

Of particular interest are the novel hybridomas CLNH5 and CLNH11, hybridomas obtained from CLNH5 and 11, antibodies derived from such hybridomas, derivatives of such antibodies and the use of the antibodies and their derivatives for diagnosis and therapy. CLNH5 and 11 are obtained by fusion between the fusion partner UC729-6 with lymphocytes from lymph node cells of a patient having cervical cancer. UC729-6 is on deposit at the A.T.C.C. with Accession No. CRL 8061. UC729-6 was deposited for patent purposes in conjunction with the filing of application Serial No. 247,652.

The lymphocytes employed for fusion were from a draining lymph node from the spinal area and peripheral blood lymphocytes from a patient having cervical carcinoma. The fusion is performed by combining the patient's lymphocytes from the lymph node with the fusion partner UC729-6 at a ratio of about 2:1 in a solution of about 35% polyethylene glycol in HEPES buffered RPMI 1640. The mixture of cells is then suspended in appropriate selective medium, particularly HAT medium containing about 10% fetal bovine serum, placed in wells at about 10^5 cells per well and a sufficient time permitted for the cells to grow. The selective medium is replaced from time to time.

Wells from the above fusion provided clones specifically reactive with the cervical cancer cells of the host patient which were designated CLNH5 and 11. These wells provided human IgM and IgG monoclonal antibodies, respectively, which react with antigen found on a variety of cervical carcinomas and other tumor cell lines, e.g., small cell carcinoma of the lung, but not with normal tissues and normal cell lines, which were tested.

The hybridomas and monoclonal antibodies can find use in a variety of ways, particularly as sources

of genetic material, as reagents, and as precursors to products which find use as reagents.

The subject hybridomas may be used as a source for genetic material. For example, the subject hybridomas may be fused with other fusion partners to provide novel hybridomas having the same secretory capabilities as CLNH5 and 11 to provide antibodies having the same specificity. Such fusions may result in the production of antibodies having different heavy chains so as to provide the other classes or subclasses of antibodies, e.g., G, A or M.

The hybridoma may also be used as a source of DNA, which by hybrid DNA technology, the genes may be excised, introduced into a lymphoma for production of the mature antibodies.

The monoclonal antibodies can be used in a variety of ways, both in vivo and in vitro diagnosis, as well as in therapy. For many applications, the antibodies will be labeled with a compound which imparts a desired property to the antibodies, such as providing a detectable signal, providing cytotoxicity, providing for localized electromagnetic radiation, or the like. Labels may include radionuclides, enzymes, fluorescers, toxins or the cytotoxic fragment of toxins, particles, metals, metalloids, etc. The antibodies may be incorporated in liposome membranes or modified with lipids, so as to be incorporated in such membranes. The antibodies by themselves or labeled, may be used in in vitro diagnosis for measuring the presence of antigens associated with a neoplasm such as cervical cancer, for in vivo diagnosis for introduction into a host, e.g., intravenously, in a physiologically acceptable carrier, e.g., PBS, or may be introduced for therapeutic purposes in the same manner.

The antibodies by themselves or labeled, may also be used for treating a neoplasm in human host such as cervical carcinoma, prostate tumor, colon carcinoma,

lung cancer, breast cancer and melanoma. The antibodies of this invention are easily soluble in physiological saline, and therefore can be injected intravenously or intramuscularly as a saline solution or a drip. Furthermore, the antibodies of the invention can be used in the form of an ointment or suppository.

The amount of antibody employed will vary depending upon the particular application. Introduction of antibodies for diagnostic and therapeutic purposes has been extensively described in literature.

The entire antibody need not be used, for many applications only a fragment having intact variable regions will suffice. For example, Fab fragments, $F(ab')_2$ fragments, or Fv fragments may suffice.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

MATERIALS AND METHODS

Fusion and Selection of Hybridomas.

Lymph nodes were teased with nugent forceps in RPMI 1640 media and isolated lymphocytes were cultured overnight at 37°C and 5% CO_2 in RPMI 1640 with 10% fetal calf serum (FCS) and 2mM L-glutamine. Lymphocytes were counted and mixed at a ratio of 2:1 with the human lymphoblastoid B cell line UC729-6 (Handley and Royston. 1982, in Hybridomas in Cancer Diagnosis and Treatment, eds. Mitchell and Oettgen, pp. 125-132, Raven Press, N.Y.), then fused with polyethylene glycol 1500 by a modification of the technique by Gefter et al., Somatic Cell Genetics (1977) 3:321-336. Fused cells were plated at 10^5 cells/well in a Costar 96 well microtiter plate with

Hypoxanthine-Amethopterin-Thymidine (HAT, Littlefield, Science (1964) 145:709-710) supplemented RPMI 1640 with 10% FCS and L-glutamine. Within 10-20 days, wells positive for hybridoma growth were assayed for human antibody production and their reactivity to a limited human cell panel by an enzyme immunoassay (EIA). Wells positive for reactivity were cloned by limiting dilution without the use of feeder layers and expanded for further study.

Enzyme Immunoassay.

Human MoAbs and their reactivity to cells were detected by a modification by an EIA previously described (Handley et al. J. of Immunologic Methods (1982) 54:291-296, as modified by Glassy et al., J. Immunologic Methods (1983) in press). Briefly, 50µl of either an affinity purified goat anti-human Ig or a $4x10^6$ target cell/ml suspension was immobilized in triplicate wells of an immunofiltration manifold. (The specifically designed microtiter plate which serves as both an incubation chamber and filtration manifold (VP no. 107; V and P Scientific, San Diego, CA). The bottom of each well contains a 0.6mm hole over which is placed a 6mm diameter glass fiber filter. Surface tension prevents fluid volumes less than 100µl from draining through the hole until a vacuum is applied. When vacuum is applied, fluid is drawn through the filter and out the drain hole leaving particulate matter trapped on the filter. After washing 3x with 0.3% gelatin in phosphate buffered saline, 50µl of hybridoma supernatant were incubated 30 min at room temperature. Filters were then washed and incubated with 50µl of a horseradish peroxidase conjugated goat anti-human Ig for an additional 30 min. Filters were washed again and incubated with 150µl of a 400µg/ml solution of ortho-phenylene diamine in citrate buffer. 100µl of each well were then transferred to a new plate

containing 50µl of 2.5M H_2SO_4 and read on a Dynatek (Alexandria, VA) MR 580 micro-ELISA reader at 492nm.

Hybridoma culture fluids were precipitated with 50% ammonium sulfate and crude Ig fractions collected. The precipitates were dissolved in phusiological saline and purified by affinity chromatography using S-aureus Protein A-bound Sepharose with IgC and Sepharose-(sheep anti(humanIgM) antibody) with IgM. From 1L of the culture fluid of CLNH5, 2.2mg IgM was obtained, while from 1L of the culture fluid of CLNH11, 3.0mg IgG was obtained.

RESULTS

Table 1 outlines the results of the fusion attempting to produce anti-SCCC (squamous cell carcinoma of cervix) human MoAbs. The fusion producing CLNH5 and CLNH11, human-human hybridomas secreting a MoIgMk and a MoIgG reactive with SCCC cell lines, generated 6 growth positive wells of 80 wells plated. Hybridomas CLNH5 and CLNH11 were cloned and expanded when found to react with the cervical carcinoma cell lines, CaSki and Hela.

TABLE 1

GENERATION AND IDENTIFICATION OF HUMAN MoAbs

Lymph Node draining	#Lympho-cytes fused	#Hybri-domas generated	#Secret-ing M	G	A	#Human reactive
Cervical Carcinoma (SCCC)	$7.0x10^6$	6	2	1	0	2 (CLNH5 and 11)

The relative amounts of human MoAb bound to each of the cell lines listed was measured by EIA.

Antibody (IgM) secreted by CLNH5 shows positive reactivity with carcinomas of the cervix (CaSki, Hela), lung (T293, Calu-1, and SK-MES-1), melanoma (SK-MEL-28), and prostate (LnCap) and was

negative for normal fibroblasts, T lymphocytes and peripheral blood lymphocytes. Antibody (IgG) secreted by CLNH11 shows positive reactivity with carcinomas of the cervix (CaSki, Hela), prostate (PC-3), breast (ZR-76-1), colon (COLO-205) and melanoma (G-361) and was negative for normal fibroblasts (WI'38 and MRC-9), T. lymphocytes and peripheral blood lymphocytes.

The cytobiochemical properties of the hybridomas of the present invention are shown below.

Hybridoma CLNH5:-

(1) Number of chromosomes: 60 to 90 (maximum frequency 80).

(2) It secretes human immunoglobulin M (IgM).

(3) Doubling time: 30-40 hours.

(4) Lymphocytic single cell.

(5) Its DNA content is at least two times, for example, 2 to 2.5 times, that of normal human lymphocytes.

(6) IgM binds to human cervical carcinoma cells and the other carcinomas mentioned above.

In addition, the above hybridoma CLNH5 can be proliferated in HAT medium (medium containing hypoxanthine, amethopterin and thymidine).

Hybridoma CLNH11:-

(1) Number of chromosomes: 60 to 90 (maximum frequency 80).

(2) It secretes human immunoglobulins G (IgG).

(3) Doubling time: 30-40 hours.

(4) Lymphocytic single cell.

(5) Its DNA content is at least two times, for example 2 to 2.5 times, that of normal human lymphocytes.

(6) IgG binds to human cervical carcinoma cells, and the other carcinomas mentioned above.

In addition, the above hybridoma CLNH11 can be proliferated in HAT medium.

The relative DNA content (the ratio to the DNA content of normal human lymphocytes) was determined by a method which comprises dyeing the hybridoma and then separating and analyzing it by a cytofluorometer.

The properties of the monoclonal human immunoglobulines in accordance with this invention are shown below.

Monoclonal Human Immunoglobulin
Produced by the Hybridoma CLNH5

(a) It is human immunoglobulin M (IgM).

(b) It has a stronger binding affinity to cell lines, Hela and CaSki, than to normal fibroblasts (WI-38).

(c) It does not react with human red blood cells, nor shows an agglutination reaction on human red blood cells.

(d) It is composed of heavy chains (H chains) and light chains (L chains), has a molecular weight of about 180,000 (monomer), and exists as a pentamer in the culture fluid.

Monoclonal Human Immunoglobulin
Produced by the Hybridoma CLNH11

(a) It is human immunoglobulin G (IgG).

(b) It has a stronger affinity to cell lines, Hela and CaSki, than to normal fibroblasts (WI-38).

(c) It does not react with human red blood cells, nor shows an agglutination reaction on human red blood cells.

(d) It is composed of H chains and L chains and has a molecular weight of about 150,000.

The binding activity of human monoclonal antibodies distinguishing neoplastic cells from normal cells was measured as follows:

An original human tissue section including carcinoma cells and normal cells was fixed on a glass plate by glutaraldehyde, and then stained by enzyme immunoassay according to the method of Sternberger et al. J. Hist. Cyto. 18 315 (1970).

The subject monoclonal antibodies are useful for the diagnosing, imaging and potentially for treating cervical carcinoma as well as other reactive tumors. Because of the specificity of the monoclonal antibodies over a rnage of cervical carcinomas from different hosts, the subject antibodies can be used in different hosts, rather than solely with the host source of the antigen. Because the subject antibodies are human, they are less likely to produce a significant immune response when employed in in vivo diagnosis or therapy.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practices within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. Human monoclonal antibodies capable of distinguishing human neoplastic cells from the corresponding normal human cells.

2. Human monoclonal antibodies according to claim 1 capable of distinguishing human solid tumor cells from the corresponding normal human cells.

3. Human monoclonal antibodies according to claim 1 capable of distinguishing human cervical carcinoma cells from normal human cervical cells.

4. Human monoclonal antibodies according to claim 3, obtained from a human hybridoma CLNH5 or hybridomas derived from CLNH5.

5. Human monoclonal antibodies according to claim 3, obtained from a human hybridoma CLNH11 or hybridomas derived from CLNH11.

6. Human monoclonal antibodies according to any one of the preceding claims 1 to 5 or fragments thereof labeled with a label capable of providing a detectable signal.

7. Human monoclonal antibodies according to claim 6, wherein said label is a radionuclide.

8. Human monoclonal antibodies according to any one of the preceding claims 1 to 5 or fragments thereof labeled with a toxin.

9. Human hybridomas having genes from the same human neoplastic host lymphocytes or human hybridomas derived therefrom.

10. Human hybridomas or human hybridomas derived therefrom according to claim 9, wherein the human neoplastic host is a human solid tumor host.

11. Human hybridomas or human hybridomas derived therefrom according to claim 10, wherein the human solid tumor host is a human cervical carcinoma host.

12. Human hybridomas CLNH5 or CLNH11 according to claim 11.

13. A method for determining the presence of a neoplasm which comprises:

combining a sample from a host suspected of having a neoplasm with monoclonal antibodies according to any one of the preceding claims 1 to 5 or fragments thereof, or said antibodies or fragments labeled with a label capable of providing a detectable signal, and

detecting the presence of the binding of said monoclonal antibodies or fragments thereof, or said antibodies or fragments labeled with a label capable of providing a detectable signal to their homologous antigen.

14. A method according to claim 13, wherein said sample is host tissue.

15. A method according to claim 13, wherein said neoplasm is a solid tumor.

16. A method according to claim 15, wherein said solid tumor is a cervical carcinoma, prostate tumor, colon carcinoma, lung cancer, breast cancer or melanoma.

17. A method for producing human monoclonal antibodies specific for neoplastic cells as distinct from normal cells and free of non-human antigens, which comprises:

fusing B-lymphocytes from human lymphnodes, human lymph glands, human bone marrow, human spleen or human blood, associated with a neoplasm in a human host with a human fusion partner to produce hybridomas;

cloning said hybridomas to produce individual clones;

screening said clones for monoclonal antibodies specific for said tumor cells and tumor cells from the same tissue from other hosts; and

growing said specific clones, whereby said monoclonal antibodies are produced.

18. A method according to claim 17, wherein said neoplasm is a solid tumor in a human host.

19. A method for producing monoclonal antibodies specific for solid tumor cells as distinct from normal cells and free of non-human antigens, which comprises:

growing cells according to claim 12.

20. A method according to claim 19, wherein said tumor is a cervical carcinoma.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US83/00781

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. CL. 435/68
INT. CL. [3] G01N 33/54

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435 / 41, 68, 172, 240, 241, 948 424 / 85, 1 436 / 548 ~~260 / 112B~~

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

BIOLOGICAL ABSTRACTS 1977-1983 HUMAN MONOCLONAL ANTIBODY OR HUMAN HYBRIDOMA AND NEOPLASM OR CANCER OR TUMOR
INDEX MEDICUS 1980-1983

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A,X	EP, A, 0044722, 27 JANUARY 1982, KAPLAN ET AL	1-20
A	GB, A, 2,086,937, 19 MAY 1982, CROCE	1-20
X,A	N, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, 77(11), NOVEMBER 1980, SCHLOM ET AL, "GENERATION OF HUMAN MONOCLONAL ANTIBODIES REACTIVE WITH HUMAN MAMMARY CARCINOMA CELLS" P. 6841-5	1-20
A	N, NATURE, 244, 17 AUGUST 1973, SCHWABER ET AL, "HUMAN MOUSE SOMATIC CELL HYBRID CLONE SECRETING IMMUNOGLOBULINS OF BOTH PARENTAL TYPES" p. 444-7	1-20
A	N, CLINICAL CHEMISTRY 27(9), SEPTEMBER 1981 BROWN ET AL, "USE OF MONOCLONAL ANTIBODIES FOR QUANTITATIVE ANALYSIS OF ANTIGENS IN NORMAL AND NEOPLASTIC TISSUES" P. 1592-6	1-20
A,X	N, FEDERATION PROCEEDINGS, 41(3), 1 MARCH 1982, GLASSY ET AL, "A HUMAN MONOCLONAL ANTIBODY REACTIVE AGAINST A HUMAN TUMOR-ASSOCIATED ANTIGEN" P. 553 ABSTRACT # 1657	1-20

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
8 SPETEMBER 1983	15 SEP 1983
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	JOHN E. TARCZA

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	N, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, 77(8), AUGUST 1980, GILLILAND ET AL, "ANTIBODY DIRECTED CYTOTOXIC AGENTS: USE OF MONOCLONAL ANTIBODY TO DIRECT THE ACTION OF TOXIN A CHAINS TO COLORECTAL CARCINOMA CELLS." P. 4539-43	8

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.